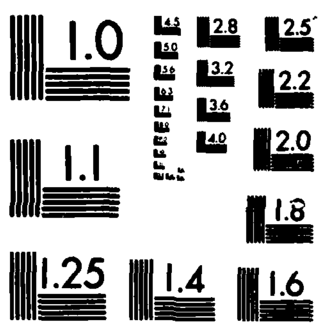
AD-A164 258 MULTIDISCIPLINARY STUDIES ON EXCITATORY AMINO ACIDS AS TRANSMITTERS IN THE BRAIN(U) CALIFORNIA UNIV IRVINE C W COTMAN 07 NOV 85 ARO-19016.14-LS DAAG29-82-K-0194 1/1

UNCLASSIFIED F/G 6/1 NL

END

FILMED



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

(2)

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: ARO 19016.14-LS	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): Multidisciplinary Studies on Excitatory Amino Acids as Transmitters in the Brain	5. TYPE OF REPORT & PERIOD COVERED: 27 Sep 82 - 26 Sep 85 Final Report
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Carl W. Cotman	8. CONTRACT OR GRANT NUMBER(s): DAAG29-82-K-0194
9. PERFORMING ORGANIZATION NAME AND ADDRESS: University of California, Irvine, CA	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: N/A
11. CONTROLLING OFFICE NAME AND ADDRESS: U. S. Army Research Office, Post Office Box 12211, Research Triangle Park, NC 27709	12. REPORT DATE: November 7, 1985
	13. NUMBER OF PAGES: 6
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

DTIC ELECTE FEB 1 2 1986 S D

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

B

18. SUPPLEMENTARY NOTES

The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Amino Acids
Brain Transmitters
Glutamate Receptors
Synaptic Plasticity
Brain Pathologies
Neurotoxicity

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This research has provided the first definition of the major receptor types at a biochemical level, showed they could be localized anatomically and described their organization in the rodent brain. Thus, these findings provide a solid foundation upon which the role of glutamate receptors in synaptic plasticity, various brain pathologies, neurotoxicity and learning and memory can be evaluated rigorously.

DD FORM 1473, 1 JAN 73 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

ARO 19116.14-LS

MULTIDISCIPLINARY STUDIES ON EXCITATORY AMINO ACIDS

AS TRANSMITTERS IN THE BRAIN

FINAL REPORT

Carl W. Cotman, P.I.

University of California, Irvine

November 7, 1985

U.S. Army Research Office

Research Agreement No. DAAG 29-82-K-0194

Approved for public release;
Distribution Unlimited

FINAL REPORT
Research Agreement No. DAAG 29-82-K-0194

A. Statement of the problem studied

Our major goal has been to analyze the excitatory amino acid receptors in the brain, particularly those mediating responses by glutamate and aspartate. Acidic amino acids, principally L-glutamate and L-aspartate, appear to be the major excitatory transmitters in the CNS. As such, these molecules may be the most abundant CNS neurotransmitter class. L-Glutamate shows many of the characteristics of other neurotransmitters. Glutamate is present in high levels in the brain and is relatively enriched in select neurons. Transmitter glutamate is supplied to nerve terminals by synthesis, primarily via the enzyme glutaminase, and by high affinity uptake. The mechanism for transmitter inactivation in the synapse appears to be the rapid removal by a high affinity uptake system. Both endogenous L-glutamate and L-glutamate derived from exogenously supplied radiolabelled precursors exhibit Ca^{++}-dependent release upon depolarization. A transport system responsible for the loading of L-glutamate into synaptic vesicles has also been identified. Excitatory amino acids act on a variety of distinct receptor types, and there are now available many compounds which can mimic or block synaptic activity at excitatory amino acid using synapses.

Our approach has involved the analysis of these receptors in isolated synaptic membranes and their further definition and anatomical localization via light microscopic autoradiography.

B. Summary of the most important results

Excitatory amino acid activity is mediated by at least four distinct receptors. Three receptor types have been identified by electrophysiological experiments wherein selective agonists are iontophoretically applied to neurons in the presence of antagonists that are also applied by iontophoresis or added to the surrounding media. The agonist-defined receptors are denoted as the N-methyl-D-aspartate (NMDA), kainate (KA), and quisqualate (QA) receptors, each referring to the agonist by which the receptor is selectively activated. The fourth receptor class is identified by the potent antagonistic action of the L-glutamate analogue L-2-amino-4-phosphonobutyrate (L-AP4) on synaptic activity at certain glutamate-using synapses.

1. We have synthesized and prepared radiolabelled APB in association with New England Nuclear. Radiolabelled 2-amino-4-phosphonobutyric acid binds to a distinct class of L-glutamate binding sites and does not exhibit appreciable binding to sites not displaced by L-glutamate. The binding affinity ($K_D = 5.1 \pm 0.4$ µM) and pharmacological profile correspond to those values obtained from physiological studies of 2-amino-4-phosphonobutyric acid inhibition of synaptic transmission, and to those values obtained in ^{3}H-L-glutamate binding assays. ^{3}H-2-amino-4-phosphobutyric acid does not exhibit significant binding to the Ca^{++}/Cl^- independent L-glutamate binding site(s), nor to the Na^+-dependent L-glutamate binding site (up to 50 mM Na^+). It now appears that the L-APB binding site in membranes is heterogeneous and further work is necessary to establish its exact identity.

2. We developed the first autoradiographic method for visualizing glutamate receptors in histological sections of brain. In the initial study, we used the hippocampus to investigate possible subpopulations of ^{3}H-L-glutamate binding sites. By using quantitative autoradiography, the pharmacological specificy of ^{3}H-L-glutamate binding in discrete terminal fields was determined. We reported that there are at least four distinct classes of ^{3}H-L-glutamate binding sites which differ in their anatomical distribution, pharmacological profile and regulation by ions. Two of these sites seem to correspond to the KA and NMDA receptor classes and a third site may represent the QA receptor. The fourth binding site does not conform to present receptor classifications. None of these binding sites corresponds to the major glutamate binding site observed in biochemical studies with isolated membrane preparations.

3. In several follow up studies, we analyzed the distribution of each receptor subtype (except the L-APB receptor) in the rodent brain via autoradiography and defined their kinetic properties and pharmacological specificity.

   a. NMDA receptors: These sites are selectively activated by NMDA and less selectively by L-glutamate, L-aspartate, and L-homocysteate. This action is most potently antagonized by the D isomer of 2-amino-5-phosphonopentanoate (D-AP5) and nearly as potently by D-2-amino-7-phosphonoheptanoate. D-α-Aminoadipate is approximately ten fold less potent. NMDA depolarizations are not antagonized by glutamate diethyl ester (GDEE). In radioligand binding experiments these receptors appear to be labelled by both ^{3}H-L-glutamate and by ^{3}H-D-AP5. Using either ligand, the highest concentrations of these sites in the rat brain are found in the CA1 field of the hippocampus. These receptors display considerable anatomical specificity. When assessed by NMDA displacement of ^{3}H-L-glutamate binding, these sites can be observed in the superficial layers of the cerebral cortex, certain amygdaloid nuclei, anterior olfactory cortex, and striatum (particularly the nucleus accumbens).

   The CA1 region of the hippocampus contains the highest density of these sites. Telencephalic regions in general have high levels of binding sites. The cerebral cortex shows significant density variations among the differing layers and regions, with the highest levels found in the frontal cortex layers I-III. Within the basal ganglia, the highest levels are found in the nucleus accumbens, intermediate levels in the caudate/putamen, and very low levels in the globus pallidus. Thalamic regions have moderate levels with variations among differing regions. Midbrain and brainstem have low levels of binding sites but within these regions there are structures exhibiting higher levels (e.g., the nucleus of the solitary tract and the inferior olive). Most but not all of the regions previously proposed to use glutamate as an excitatory transmitter contain a moderate to high density of NMDA sites. Thus, the distribution of NMDA-sensitive ^{3}H-L-glutamate binding sites suggests that the NMDA receptor represents a major, distinct subset of excitatory amino acid receptors and indicates regions in which neurotransmission may be mediated or modulated by this receptor.

   b. Kainate receptors: These receptors are activated by kainate (KA) and domoate. Excitation of this receptor is antagonized by D-γglutamylglycine (DGG), γ-glutamylaminomethylsulfonate (GAMS), and other recently identified antagonists. It should be noted that although these antagonists also inhibit NMDA responses, KA responses appear to represent distinct receptors since the selective NMDA antagonists do not inhibit KA-induced depolarizations.

In radioligand binding experiments these sites are readily labelled by ^{3}H-KA. This ligand differentiates two binding sites with high and low affinities. These two sites apparently have a similar pharmacological profile, and are both thought to represent the KA receptor. Recent evidence has also indicated distinct binding site populations based upon differing association and dissociation rate kinetics. Using autoradiographic techniques, ^{3}H-KA binding sites have been found to display a marked anatomical specificity. The most dense localization of these sites is in the stratum lucidum of the hippocampus. This observation is noteworthy because this region is also one of the most sensitive regions in the CNS to the excitotoxic actions of KA. Analysis of the KA receptor function in this pathway should yield valuable insights to the normal function of this receptor. In other regions of the brain, KA binding sites are preferentially found in the caudate/putamen, deep layers of neocortex, and in the hypothalamus. Together with the observation that these binding sites are enriched in purified synaptic junctions, these results suggest that KA receptors are not simply a non-specific excitotoxin receptor but a distinct, pathway-specific receptor.

c. Quisqualate receptors: Quisqualate and amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) selectively activate the third class of excitatory amino acid receptor. Weak antagonism is displayed by GDEE and cis-2,3-piperidine dicarboxylic acid (PDA). In general, however, these antagonists are neither potent nor selective. These receptors have been reported to be labelled by a variety of radioligands. It appears that QA has a high affinity for a variety of binding sites, but to date, only the binding site also labelled by ^{3}H-AMPA displays the appropriate pharmacological profile. The development of more potent and selective antagonists against QA and AMPA would be useful for confirming the identity of the ^{3}H-AMPA binding site. ^{3}H-AMPA sites also display considerable regional variation when assayed by autoradiographic techniques.

These binding sites are found in high concentrations in the hippocampus, cerebral cortex (especially layers I-III), induseum griseum and dorsal lateral septum. Intermediate concentrations are found in the corpus striatum and deeper layers of cerebral cortex; and lower concentrations are found in the diencephalon, midbrain and brainstem. These results demonstrate that ^{3}H-AMPA binding sites are found throughout the CNS and suggest brain regions which may use quisqualate receptors as glutamate neurotransmitter receptors.

The pharmacological properties of these sites correspond to those studied by electrophysiological methods. The three most potent and well characterized QA receptor agonists (QA, AMPA and L-glutamate), are the most potent displacers of AMPA binding to tissue sections. Also in accord with physiological studies, compounds which preferentially act at the NMDA receptor are not potent at this site (D-α-aminoadipate, D-2-

amino-5-phosphonovalerate, D-aspartate, 2-amino-7-phosphonoheptanoate and NMDA). In addition, KA is found to be greater than 100-fold more potent as a displacer of ^{3}H-KA binding than ^{3}H-AMPA binding. Thus, binding in tissue sections has the pharmacological specificity expected for the AMPA/QA receptor.

Currently, the KA and QA receptors are both good candidates for the receptor(s) mediating the fast EPSP response in a number of excitatory amino acid pathways. The general excitatory amino acid antagonists DGG, PDA, GAMS, and kynurenate, block the evoked synaptic response while the selective NMDA antagonists do not inhibit the responses. In general, KA and QA have been found to have similar pharmacological profiles. It is usually the case that if an antagonist blocks the responses to QA then it will block the responses to KA to a slightly greater extent. Evidently, the structural specificity requirements for these two receptors are relatively similar. The nonselective excitatory amino acid antagonists are effective against synaptic responses in the spinal cord, the caudate nucleus, dentate gyrus, hippocampal CA1 and CA3 fields, retina, and nucleus of the solitary tract. Since the potency of the available antagonists for the synaptic receptor is not remarkably different from that against KA and QA responses, it is not possible at present to define precisely the receptor mediating the synaptic response.

Conclusion

In the past several years we provided the first definition of the major receptor types at a biochemical level, showed they could be localized anatomically and described their organization in the rodent brain. Thus, these findings provide a solid foundation upon which the role of glutamate receptors in synaptic plasticity, various brain pathologies, neurotoxicity and learning and memory can be evaluated rigorously.

As the acidic amino acids L-glutamate, L-aspartate, and others play a central role in intermediary metabolism, as well as serving as transmitters, they present special difficulties in identifying the neurons which use these compounds as neurotransmitters. Thus, unlike other neurotransmitters such as acetylcholine, determination of L-glutamate and its synthetic enzymes in a neuron does not, in itself, indicate that this cell uses glutamate as a neurotransmitter. Thus, studies of glutamate levels, enzyme activities, and uptake, are complicated by the metabolic role of L-glutamate. Consequently, despite the strong evidence for glutamate mediated neurotransmission, it is difficult, and not always feasible, to definitively establish the use of L-glutamate as a neurotransmitter in a given neuronal population. Thus, the identification of the excitatory amino acid receptor used at a given synapse is a critical and straightforward test for establishing that an acidic amino acid is used at that synapse. With the difficulty in demonstrating that specifically L-glutamate, L-aspartate, or a closely related derivative (L-homocysteate, L-cysteate, cysteine sulfonate, and others) is the transmitter in a given synapse, it is now possible to establish the identity of the excitatory amino acid receptor used without knowing the identity of the transmitter.

Recent progress in the characterization of the excitatory amino acid receptors, and development of better antagonists will lead to a better understanding of the in vivo functions of these receptors, their mechanisms of action (e.g. second messenger systems) and the development of defensive posture against neurotoxic agents with similar structure reproperties.

C. List of all publications

1. Monaghan, D.T., D. Yao, and C.W. Cotman, L-^{3}H-Glutamate binds to kainate-, NMDA-, and AMPA-sensitive binding sites: an autoradiographic analysis. Brain Res. 340: 378-383, 1985.

2. Mena, E.E., Whittemore, S.R., Monaghan,D.T. and Cotman, C.W. Ionic regulation of glutamate binding sites. Life Sciences, 35: 2427-2433, 1984.

3. Mena, E.E., Monaghan, D.T., Whittemore, S.R. and Cotman, C.W. Cations differentially affect subpopulations of L-glutamate receptors in rat synaptic plasma membranes. Brain Research, 329: 319-322, 1985.

4. Monaghan, D.T. and C. W. Cotman, Anatomical organization of NMDA, Kainate and Quisqualate receptors. In: Excitatory Amino Acids, Edited by P.J. Roberts, J. Storm-Mathisen, and H. Bradford, MacMillan Press, London (in press).

5. Cotman, C.W. and D.T. Monaghan, Anatomical organization of excitatory amino acid receptors: An overview. Epilepsia (in press).

6. Mena, E.E. and Cotman, C.W., Pathological levels of ammonium ions block L-glutamate uptake. Experimental Neurology 89: 259, 1985.

7. Monaghan, D.T., Yao, D., Olverman, H.J., Watkins, J.C. and Cotman, C.W. Autoradiography of D-2-^{3}H Amino-5-phosphonopentanoate binding sites in rat brain, Neuroscience Letters, 52: 253-258, 1984.

8. Monaghan, D.T. and Cotman, C.W. Distribution of N-Methyl-D-Aspartate-Sensitive L-^{3}H-glutamate binding sites in rat brain. Journal of Neuroscience in press.

9. Monaghan, D.T., Yao, D., and Cotman, C.W. Distribution of ^{3}H-AMPA binding sites in rat brain as determined by quantitative autoradiography. Brain Research 324: 160-164, 1984.

10. Mena, E.E., Monaghan, D.T., Whittemore, S.R. and Cotman, C.W. Cations differentially affect subpopulations of L-glutamate receptors in rat synaptic plasma membranes. Brain Research, 329: 319-322, 1985.

11. Altschuler, R.A., Monaghan, D.T., Haser, W.G., Wenthold, R.J., Curthoys, N.P., and Cotman, C.W. Immunocytochemical localization of glutaminase-like and aspartate aminotransferase-like immunoreactivities in the rat and guinea pig hippocampus. Brain Research, 330: 225-233, 1985.

12. Monaghan, D.T., Holets, V., Toy, D. and Cotman, C.W. Anatomical distributions of four pharmacologically distinct 3H-L-glutamate binding sites. Nature, 306: 176-179, 1983.

13. Monaghan, D.T., McMills, M.C., Chamberlin, A.R., and Cotman, C.W. Synthesis of ^{3}H 2-Amino-4-phosphonobutyric acid and characterization of its binding to rat brain membranes: a selective ligand for the chloride/calcium dependent class of L-glutamate binding sites. Brain Research, 278: 137-144, 1983.

14. Whittemore, S.R., Mena, E.E., Monaghan, D.T. and Cotman, C.W. Regional distribution and ionic requirement of Cl-/Ca^{2+}-Activated and Cl-/Ca^{2+}-Independent glutamate receptors in rat brain. Brain Research, 277: 99-107, 1983.

D. List of participating scientific personnel

Carl Cotman
Richard Bridges
Dan Monaghan - Ph.D. awarded
Deborah Yao
Scott Whittemore
Eric Harris

END

FILMED

3-86

DTIC